J. Chem. & Cheml. Sci. Vol.1 (1), 21-28 (2010)

Volumetric Method for Determination of Coenzyme Q10 in Pharmaceutical Samples with Ascorbic Acid

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ABSTRACT

An accurate, simple and precise method has been developed for the determination of Coenzyme Q10 (Ubiquinone) in pharmaceutical preparations by titrating it against standardized ascorbic acid in slightly acidic medium (pH 5.0) using starch as indicator. The reaction stoichiometry was found to be 1**:**1 (Coenzyme Q10: Ascorbic acid). The method reported gives accurate and reproducible results in the range 3.55 X 10^{-2} mg to 52.53 X 10^{-2} mg, with standard deviation being ± 0.017 whereas the coefficient of variation was ± 3.4%. The proposed method is relatively free from the interference caused by metallic excipients, Fe(II), Cd(II), Co(II), Fe(III), Cu(II), Zn(II), Mg(II), Pb(II), Cr(II) and Ni(II), commonly found in the pharmaceutical samples. Moreover interference caused by other antioxidants N-acetyl –L cysteine, lipoic acid, glutathione, vitamin A, vitamin E, vitamin B_6, B_2 and B_1 was also studied.

INTRODUCTION

Free radicals are highly reactive ions containing one or more unpaired electrons. Free radicals are like unguided missiles that bounce around and attack healthy cells. The cell membranes are teared off and the cells get infected[1]. Consequently the destruction of integrity of genetic codes starts causing mutations[2] such as cancer or similar degenerative changes. Free radical scavangers also known as antioxidants are natural protectors from assaults by free radicals. They protect our cells from unhealthy oxidation by offering easy electron target for free radicals. Free radical scavengers thus deenergize or stabilize the lone free radical electrons. Antioxidants protect the body cells from free radicals before they cause mutation instead of repairing the damage afterwards.

The two most well known antioxidants are Coenzyme Q10 and Vitamin C. Coenzyme Q10 (Ubiquinone) is a member of quinone family. It consist of a “quinone” nucleus in which two of the four hydrogen atoms are each replaced by a methoxy goup (MeO), one by a methyl group (Me) and the fourth is replaced by a chain of ten isoprene units(this is why it is designated as Q10)[3]. (Fig.1)

It is a vitamin like nontoxic membrane soluble cofactor that can take up one or two electrons as well as an appropriate number of protons (Fig 2).

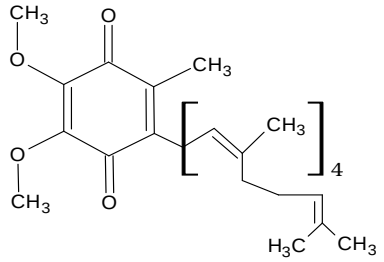

Fig.1: Coenzyme Q10 molecule

It plays a critical role in generating energy within the mitochondria[4], help in transmit protons across membrane during an oxidation reduction cycle[5] , act as free radical scavenger[6] that is profoundly protective against myocardial, cerebral, renal, genetic disorders, skeletal muscle ischemia and neurodegenerative disorders[7] and even AIDS[8]. Determination of Coenzyme Q10 by HPLC method in blood samples (upto 0.65 μg/ml)[9], in biological materials [10], in plasma samples[11] (0.79 ± 0.23 μg/ml and 0.26- 1.03 mg/l) and in serum samples (1.28 μg/ml) are already developed. HPLC technique with UV-Detection[12] for the amount of coenzyme Q10 in selected food items gave 3-5mg of Q10 per day in food and the effect of cooking was a 14-32% destruction of coenzyme Q10. Simultaneous determination of tissue tocopherols, tocotrienols, coenzyme Q10 and ubiquinols was carried out using gradient high pressure liquid chromatography (HPLC)[13]. Ubiquinone Q10 concentration was highest in kidney (301± 123 nmol/g) and in heart (244 ± 22 nmol/g). Improved high–performance liquid chromatographic method for the determination of coenzyme Q10 in plasma was reported by Grossi and coworkers[14] using C18 reversed phase column, UV detection at 275 nm and extraction was done with n-hexane. The average level was found to be 0.80±0.20 mg/l, minimum detectable plasma level was 0.05 and 0.005 mg/l, recovery percentage (99± 1.2%). Individual homologues of coenzyme Q10 were determined in biological materials using a high sensitive multiple wavelength detector or an electro-chemical detector[15].

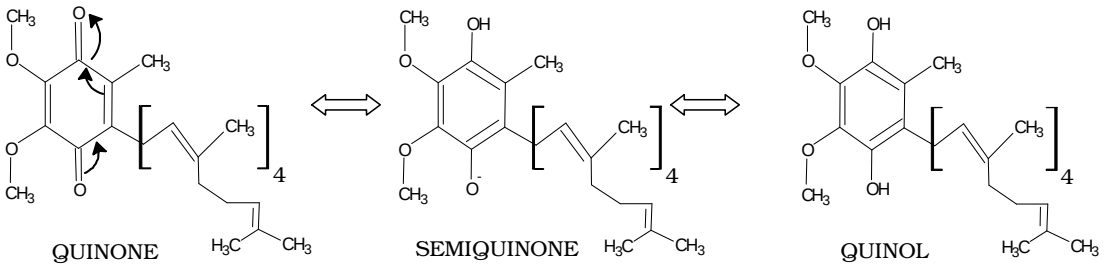

Fig.2 :Coenzyme Q10 nature

Ascorbic acid or Vitamin C ,which is structurally similar to sugar molecules, is distributed to tissues in the blood plasma in which it is one of the most important water soluble antioxidant. Eminent scientist from Haworth and coworkers in 1932 proposed

molecular formula of vitamin C as $C_6H_8O_6$. Since this substance healed scurvy, it was called 'ascorbic acid' (from the Latin 'scorbut' i.e. scurvy). Ascorbic acid is an unsaturated γ-lactone. It has an acidic taste. Ascorbic acid behaves as a monobasic acid. Its acidic properties are due to the hydrogen of the hydroxyl group in position three, and it is this hydrogen that forms salts. Reducing property of ascorbic acid is due to presence of 1,2-enediol functional group, which is also responsible for the physiological activity of ascorbic acid. It is easily soluble in water (about one gram in 8.0 ml of water), soluble in alcohol and insoluble in ether, benzene and chloroform. Antioxidant activity of ascorbic acid is owing to its high reducing potential of carbon-carbon double bond. Antioxidant activity of ascorbic acid is owing to its high reducing potential of carbon-carbon double bond. This C=C readily donates one or two hydrogens to a variety of oxidants (including oxygen free radicals, peroxides and superoxides)[16]. Ascorbic acid behaves as an efficient antioxident in several different ways, for instance by scavenging radicals produced by certain drugs, protecting lung fluids from the damages due to particularly dangerous air pollutants such as O_3 and $NO_2^{\cdot}$, reducing lipid peroxidation in cigarette smoke, and scavenging peroxyl, thiol, sulphenyl, urate, nitroxide and other radicals [17]. Ascorbic acid as antioxidant is used in treatment of diabetes, atherosclerotic coronary artery disease (CAD), sunburns, cancer, tumors and also autoimmune disorders. Owing to the wide spread use of ascorbic acid many analytical techniques are available for its determination in different matrices and at different levels. Titremetric method for the determination of ascorbic acid include various oxidizing agents such as iodine, 2,6-dichlorophenolindophenol, N-bromosuccinimide, ferric salts, cerium (IV) sulphate, chloranil , chloramine-T, bromine.

In the present work, a new rapid, reproducible and sensitive method for volumetric determination of antioxidant drug coenzyme Q10 with ascorbic acid is described. The methods involve 1:1 quantitative redox reaction between coenzyme Q10 and ascorbic acid.

EXPERIMENTAL

Reagents:

Stock solution of ascorbic acid was prepared using ascorbic acid tablets (brand name Ascorbic Acid of Unicure Laboratories Pvt. Ltd , Noida) having 100mg ascorbic acid per tablet. Accurately weighed one tablet equivalent to 100mg of ascorbic acid was thoroughly mixed with 50ml of double distilled water. The volume was made upto 250ml by adding distilled water. The resulting solution was filtered through Whatman filter paper no.42 to avoid impurities. Standardization of the solution was done iodimetrically.

For Coenzyme Q10 stock solution, Coenzyme Q10 capsules (brand name UBI-Q of Fourrts Laboratories Pvt. Ltd , Tamil Nadu) was considered having 30mg coenzyme Q10 per capsule. Accurately weighed one capsule powder equivalent to c.a. 30mg of coenzyme Q10 was thoroughly mixed with 30ml of warm double distilled water. The volume was made upto 100ml by adding distilled water. The resulting solution was filtered through Whatman filter paper no.42 to avoid impurities.

Procedure:

Different aliquots from 1.0 ml to 10.0 ml, containing 3.55×10^{-2} mg to 52.53×10^{-2} mg of drug coenzyme Q10 were taken in 150ml Erlenmeyer flask. To each of this

aliquot 0.5ml KI 10% solution and 2ml of 2N hydochloric acid was added. The colour of the solution turns light yellow. The contents of the flask were titrated against standardized ascorbic acid solution using starch as indicator. On adding starch the colour of the solution turns violet and end point is signaled by the disappearance of violet colour. Addition of ascorbic acid must be slow with constant shaking to get accurate end point.

RESULT AND DISCUSSION

In the reported titration reaction the strong reducing property of ascorbic acid has been exploited for the determination of coenzyme Q10. In the proposed method coenzyme Q10 has been determined using, standard ascorbic acid as reagent. Ascorbic acid acts as a reducing agent and donates two H^+ ions to the coenzyme Q10 molecule. The reaction was carried out in acidic medium and I^- ions added in the starting of the reaction were generated back when ascorbic acid reacts with Coenzyme Q10 i.e.I_2 is generated in situ. The stoichiometry for the reaction was found to be 1:1 for Coenzyme Q10: Ascorbic acid. The oxidation half reaction and reduction half reaction involved are given in fig 3.

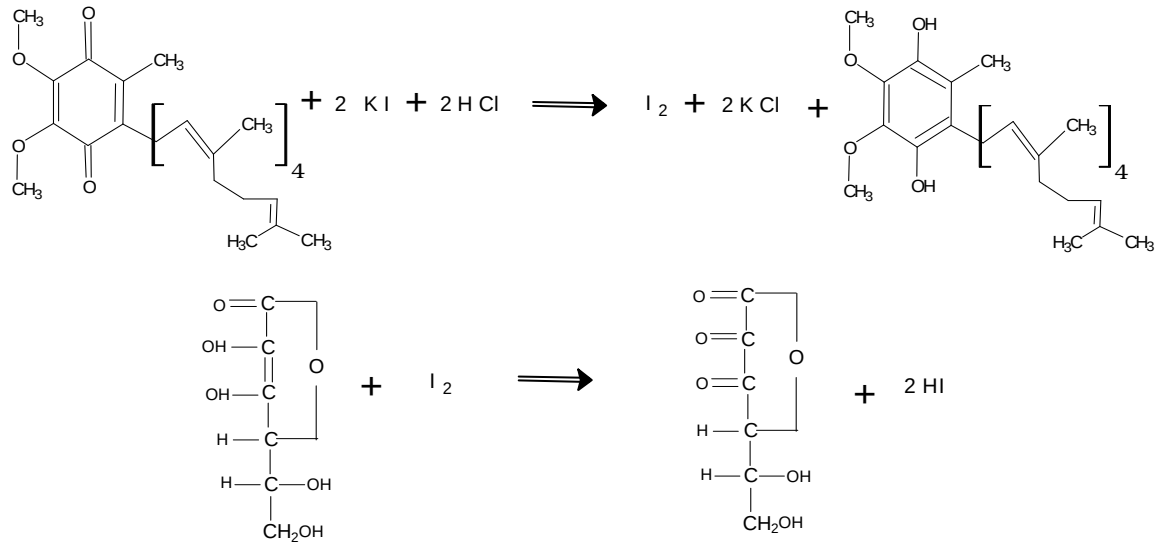

Fig. 3 : Oxidation and reduction half reactions

The net reaction involved during volumetric determination of Coenzyme Q10 with Ascorbic acid is given in fig 4.

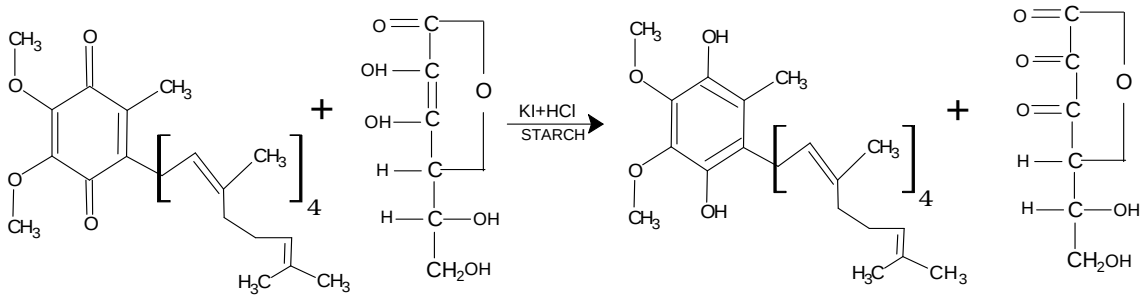

Fig. 4 : Coenzyme Q10 : Ascorbic acid 1 : 1 reaction

Various factors suitable for the titration of Coenzyme Q10 with ascorbic acid were studied. The titration of Coenzyme Q10 with ascorbic acid was studied in the temperature range 20 - 90 ^{o}C. It was found that the reaction is fast with distinct colour change at the end point occurs when the temperature is between 25-40^{o}C. At temperature higher than 40^{o}C turbidity appears and non-reproducible results are obtained. Thus further experiments were performed in the temperature range 25-40 ^{o}C. An investigation of the effect of reaction time showed that accurate results are obtained when titration is carried out at a rate 15- 20 drops per min. The rate of flow of ascorbic acid (titrant) is adjusted so that 15-20 drops are added to the mixture in Erlenmeyer flask in one minute. Contents of the flask are mixed well after addition of each drop of titrant by shaking. The end point is detected by complete disappearance of violet colour for more than 5 mins. Coenzyme Q10 solution is slightly acidic in pH (= 5.0). During its titration with ascorbic acid 2N hydrochloric acid is added to it, for the liberation of iodine. The pH of the ascorbic acid used as titrant was 5.0. On the basis of solubility of coenzyme Q10, its optimal stability and intensity of the colour change during titration, distilled water was used as the solvent.

The proposed method is accurate, rapid, precise and least time consuming. It can be used successfully for the determination of Coenzyme Q10 in the range 3.55 X 10^{-2} mg to 52.53 X 10^{-2} mg with standard deviation being ± 0.017 and coefficient of variation found to be ± 3.4%. The results are summarised in table–1.

Analysis of mixture containing binders is relatively more complex in view of the fact that large number of organic substances exists in it. Numerous methods are known which are applicable to the analysis of pure compounds but which cannot be extended to the analysis of mixtures having binders or ingredients. Normally such an approach may involve either a separatative step by means of solvent extraction, chromatography etc. or by using masking reagents.

The interference caused by various metal ions commonly found as excipents, on the titremetric determination of coenzyme Q10, was examined. Since coenzyme Q10 is found in combination with various other antioxidants in pharmaceutical preparations. Interference caused by different antioxidants commonly found in combination with coenzyme Q10 was also studied separately.

The tolerable amount of diverse metal ions and antioxidants in ppm in 1ppm of coenzyme Q10 has been determined. The influence of excipients addition on assay coenzyme Q10 and overall recovery percentage of the drug has been estimated, the results are summarised in table-2.

TABLE –1: VOLUMETRIC DETERMINATION OF COENZYME Q10 WITH ASCORBIC ACID

Coenzyme Q10 Taken (in mg)	**Coenzyme Q10 Found (in mg)**	**Number of determinations**	**Standard Deviation (S)**	Coeff. of variation **(%)**
3.55×10^{-2}	3.68×10^{-2}	10	10.64×10^{-3}	2.3%
5.43×10^{-2}	56.8×10^{-2}	10	1.23×10^{-3}	1.6%
$7.31X \times 10^{-2}$	7.45×10^{-2}	10	0.70×10^{-3}	0.9%
9.62×10^{-2}	9.54×10^{-2}	10	2.56×10^{-3}	1.5%
11.92×10^{-2}	12.02×10^{-2}	10	2.50×10^{-3}	2.0%
13.01×10^{-2}	13.10×10^{-2}	8	6.25×10^{-3}	1.8%
13.92×10^{-2}	14.20×10^{-2}	8	1.40×10^{-3}	0.9%
15.82×10^{-2}	15.80×10^{-2}	8	5.26×10^{-3}	0.7%
17.71×10^{-2}	17.79×10^{-2}	10	1.05×10^{-3}	0.6%
21.35×10^{-2}	21.40×10^{-2}	10	7.51×10^{-3}	1.9%
24.99×10^{-2}	23.86×10^{-2}	10	5.64×10^{-3}	2.3%
26.26×10^{-2}	26.30×10^{-2}	10	3.84×10^{-3}	2.1%
27.54×10^{-2}	27.83×10^{-2}	10	1.45×10^{-3}	0.5%
28.77×10^{-2}	28.76×10^{-2}	8	8.35×10^{-3}	1.5%
30.00×10^{-2}	30.01×10^{-2}	9	6.20×10^{-3}	2.1%
32.22×10^{-2}	32.37×10^{-2}	10	5.67×10^{-3}	1.4%
34.43×10^{-2}	34.45×10^{-2}	10	10.20×10^{-3}	2.7%
38.95×10^{-2}	39.00×10^{-2}	10	7.32×10^{-3}	1.6%
43.47×10^{-2}	43.50×10^{-2}	10	5.94×10^{-3}	0.8%
52.53×10^{-2}	49.04×10^{-2}	9	17.40×10^{-3}	3.4%

TABLE –2: EFFECT OF DIVERSE IONS AND ANTIOXIDANTS ON THE VOLUMETRIC DETERMINATION OF COENZYME Q10 WITH ASCORBIC ACID

Excipients	Added As	Wt. of Coenzyme Q10 Taken (in mg)	Wt. of Coenzyme Q10 Found (in mg)	Coenzyme Q10 : Excipient ratio (in ppm)	% Recovery
N-Acetyl-L-cyteine	Pure NAC	0.6035	0.5796	1 : 4.056	98.2 %
α-Lipoic acid	-	0.5552	0.4982	1 : 6.905	85.7%
Tochopherol	Vit E cap	0.6035	0.6035	1 : 2.852	100.0%
Retinol	Vit A cap	0.6035	0.6035	1 : 2.371	100.0%
Thiamine	Vit B1 tab	0.4828	0.4587	1 : 0.206	98.5%
Riboflavin	Vit B2 tab	0.4828	0.4820	1 : 0.207	99.8%
Pyridoxime	Vit B6 tab	0.4828	0.4707	1 : 0.083	99.3%
Glutathione	Pure gluta	0.5311	0.5069	1 : 0.764	98.6%
Fe(II) ion	$FeSO_4$	0.7484	0.7363	1 : 1.152	99.7%
Cd(II)ion	$CdCO_3$	1.0622	0.9656	1 : 0.286	99.2%
Co(II) ion	$CoSO_4$	0.4828	0.4587	1 : 0.932	97.8%
Fe(III) ion	(NH_4) $FeSO_4$	0.5310	0.5069	1 : 0.799	97.4%
Cu(II) ion	$Cu\ SO_4$	0.5311	0.5190	1 : 3.309	98.6%
Zn(II) ion	$ZnSO_4$	0.6035	0.5914	1 : 1.667	99.3%
Mg(II)ion	MgO	0.5311	0.4587	1 : 0.134	98.8%
Pb(II) ion	$Pb(NO_3)_2$	0.6518	0.6276	1 : 1.098	97.8%
Ni(II) ion	$NiSO_4$	0.6035	0.6035	1 : 0.930	100.0%
Cr(IV) ion	CrO_3	0.6035	0.6028	1 : 3.412	100.0%

The drug coenzyme Q10 has been successfully determined in presence of these excipients without involving any masking or pre-separative step. The limits of tolerance of interference were observed from five adjuvants in coenzyme Q10 samples and was found to be high. Recoveries were 85.7-100.0 % . Thus the method proposed can be successfully applied for routine analysis of coenzyme Q10 in pharmaceutical samples.

REFERENCES

1. Schiller H. J., *Eur.J.Biochem*, 215: 213-219 (1993).
2. Cheng K.C., *J.Bio.Chen*, 267:166-172 (1992).
3. Gian Paolo Littarru; *Casa Editrice Scientifica Internazionale*, 1-91 (1994).
4. Ernster L; Nelson BD ; *J.Biomed. & Clin.Aspects of Coenzyme Q*, 3:159-168 (1981).

5. Lenaz G;Degli Esposti M; Fato R, Cabrini L; *Biomed. and Clin. Aspects of coenzyme Q*, 3,169-82 (1981).
6. Ernster L.; Folkers K.;Yamamura Y.(eds), *Elsevier, Amsterdam*;15-8 (1997).
7. Rossi E.; Lombardo A.; Testa M.; Lippa S.; Oradei A.; Littarru G.P.; Lucente M.; Coppola E.; Manzoli U; *Biomedical and Clin.Aspects of Coenzyme Q*; 6 : 312-26 (1991).
8. Folkers K.; Brown R.; Judy W.V.; Morita M.; *Biochem. Biophys. Res. Comm*., Ms. No. G-8658 (1993).
9. Folkers K; Ye CQ; Tamagama H; Pfeiffer C; *Biofactors, Unique Identifier*: AIDSLINEMED/89351517; 1(4), 303-6 (1988).
10. Ade K; Katayama K; Ikenoya S; Nakamura T; *Biomed. And Clin. Aspects of CoQ*; 3, 53-66 (1981).
11. Kishi T; Okamoto T; Kanamori N; Folkers K; Yamagami T; Kishi H; Okada A; *Biomed. and Clin. Aspects of Co Q*;3, 67-78 (1981).
12. Weber C; Bysted A; Helmer G; *Int J Vitam Nutr Res*; 67, 2, 123-9 (1997).
13. Podda M; Weber C; Traber MG; Packer L; *J. Lipid Res*; 37, 4, 893-901 (1996).
14. Grossi G; Bargossi Am; Fiorella PL; Piazzi S; *J Chromatogr*.; 593, 1-2, 217-26 (1992).
15. Abe K; Katayama K; Ikenoya S; Nakamura T; Takada M; Yuzuriha T; Hanamura K; Yusuda K; Watson B; Kogure K; *Biomed. and Clin. Aspects of Co Q*; 3;53-66 (1981).
16. Mehlhorn. R. J., *J. Biol. Chem*; 266, 2724-31 (1991).
17. Rose,R,C., Bode AM; *Biology of free radicals scavengers an evaluation of ascorbate*. FASEB J7; 1135-42 (1993).